Journal of Crustacean Biology

The Crustacean Society

Journal of Crustacean Biology

Journal of Crustacean Biology (2017) 1–7. doi:10.1093/jcbiol/rux051

Density and gender segregation effects in the culture of the caridean ornamental red cherry shrimp *Neocaridina davidi* Bouvier, 1904 (Caridea: Atyidae)

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(Received 15 March 2017; accepted 4 May 2017)

ABSTRACT

The effect of density on growth, sex ratio, survival, and biochemical composition of the red cherry shrimp, *Neocaridina davidi* Bouvier, 1904, was studied to determine optimum rearing conditions in this ornamental species. It was tested whether gender segregation affected growth and survival of the species. To test the effect of density (Experiment 1), hatched juvenile shrimp were kept at three different densities: 2.5, 5, and 10 individuals l^{-1} ($D_{2.5}$, D_5 and D_{10}, respectively). To test the effect of gender segregation (Experiment 2), 30-day juveniles were reared in three conditions: culture with only females, culture with only males, and mixed culture (females: males 1:1) at 5 individuals l^{-1} density. Experiments lasted 90 days, and shrimp were weighted either every 30 days (Experiment 1) or 15 days (Experiment 2). At day 90, females kept at $D_{2.5}$ weighted 45% more than females stocked at D_{10} ($P < 0.05$), whereas females from D_5 did not differ from those of other densities ($P > 0.05$). Males at $D_{2.5}$ weighted 29% more than D_5 and D_{10} ($P < 0.05$). Survival was high and unaffected by treatment. Sexual differentiation did not differ among treatments. Females from $D_{2.5}$ had the lowest lipid and protein content, which would occur if they had a higher spawning. Males from $D_{2.5}$ had higher content of proteins, probably due to their larger size. Gender segregation had no effect over growth and survival; females grew up to a larger size than males both in monosex and mixed culture. It was shown that given to their non-aggressive behavior, *N. davidi* is tolerant to a high-density condition, which makes it feasible as an ornamental species.

Key Words: aquaculture, biochemical composition, growth, rearing conditions, survival

INTRODUCTION

Keeping of aquarium species has become very popular around the word and nowadays is becoming increasingly attractive. Ornamental organisms support a multi-million-dollar global industry that provides marine and freshwater aquarist with an assortment of over 1,400 species of invertebrates, vertebrates, plants, and algae (Baeza & Behringer, 2017). In contrast to the ornamental fish trade, decapod crustaceans such as shrimps, crayfishes, and crabs are relatively new to the pet trade. Nevertheless, the popularity of ornamental shrimps in freshwater aquaria has rapidly increased in recent years and may represent an entry pathway for emerging pathogens (see Patoka *et al.*, 2016).

Freshwater shrimps belonging to the family Atyidae have become very popular as algae eaters and for their attractive coloration (Heerbrandt & Lin, 2006; Turkmen & Karadal, 2012). *Neocaridina davidi* Bouvier, 1904 is a freshwater shrimp native to Asia that inhabits streams and lakes. It has an average length of 2–2.5 cm. Its sexual dimorphism is evident, females being larger than males and more brilliantly colored than males. The popular name "red cherry shrimp" accounts for the bright-red color of females. As in many freshwater species, it has direct development. This condition, added to their gregarious behavior, facilitates breeding. As a result of its uncomplicated rearing, *N. davidi* has become a frequent choice among aquarists as well as researchers (see Tropea & López-Greco, 2015) although the release into the environment through carelessness on the part of aquarists

may have ecologically important consequences, including its rapid dispersion and its negative impact on populations (see Weber & Traunspurger, 2016).

Recent studies have addressed several aspects of the reproduction and development of *N. davidi* (Tropea *et al.*, 2015; Tropea & López-Greco, 2015; Sganga *et al.*, 2016), including nutritional vulnerability of juveniles (Pantaleão *et al.*, 2015a, b). Regardless of its popularity, basic knowledge of the biology and optimal rearing conditions of *N. davidi* is lacking. It is particularly important to understand its requirements for culture to succeed. Some of the parameters that have been considered are density (Palma *et al.*, 2009), diet (Calado *et al.*, 2005b), vulnerability to disease (Bondad-Reantaso *et al.*, 2005; Bondad-Reantaso & Subasinghe, 2008), gender segregation (Kunda *et al.*, 2009), pH (Chen & Chen, 2003), salinity (Wasielesky *et al.*, 2003), substrate (Tidwell *et al.*, 1998), and temperature (Thomas *et al.*, 2000) among others.

Density is one of the key parameters to take into account to maximize the return of investment during culture. Overcrowding leads to a decrease in growth rates (Liu *et al.*, 2014; Golubev *et al.*, 2016), or an increase prevalence of diseases (Noga, 2010). Low density would denote that the system has not reached its full potential. Neither option is favorable for a producer of ornamental species, hence it is important to understand how density affects breeding. In species with a pronounced sexual dimorphism such as the caridean shrimp *Macrobrachium rosenbergii* (De Man, 1879), gender segregation (also known as "monoculture") has proven to be an effective measure to increase production and reduce the negative effect of the reproductive-dominance hierarchy in males (Ra'Anan & Sagi, 1985; Mohanakumaran Nair *et al.*, 2006).

This study aimed to investigate the effects of density and gender segregation on the growth and survival of *N. davidi*. Also investigated was how density affected the biochemical composition (total glycogen, lipids, and protein content) for both sexes of this species.

MATERIALS AND METHODS

Broodstock maintenance and production of juveniles

The reproductive stock was obtained from a commercial supplier (Acuamanus Aquarium, Buenos Aires, Argentina). Approximately 20 adult females and 20 adult males were randomly selected from the stock and placed in an aquarium (0.33 × 0.25 × 0.19 m) containing 8 l of water, and nearly 1.6 g of *Vesicularia* sp. ("Java moss"), supplied as substrate and shelter. Once females became ovigerous, they were isolated in smaller recipients (0.18 × 0.12 × 0.12 m) containing 2 l of water until juveniles hatched; newly hatched or 30-day old juveniles were assigned to the experimental trials.

All shrimp employed (including those from the stock) were kept under the same rearing conditions: dechlorinated tap water (pH 7.5, hardness 80 mg l^{1} as $CaCO_3$ equivalents), temperature was set at 27 ± 1 °C by the use of water heaters (100 W, precision 1 °C), photoperiod of 14 light:10 dark, and continuous aeration. They were daily fed *ad libitum* with tropical-fish, balanced food (Tetracolor®, Tetra, Melle, Germany), with an approximate composition of 47.5% minimum crude protein, 6.5% minimum crude fat, 2.0% maximum crude fiber 6.0% maximum moisture, 1.5% minimum phosphorus, and 100 mg kg^{-1} minimum ascorbic acid. These culture conditions were based on Tropea *et al.* (2015).

Effect of density

The effect of density (Experiment 1) was evaluated by applying three different stocking densities: 2.5, 5, and 10 shrimp l^{-1} (hereinafter referred to as $D_{2.5}$, D_5, and D_{10}, respectively). Newly-hatched juveniles were randomly assigned to a treatment and placed in small recipients (experimental unit) each containing 2 l water and 0.7 g of *Vesicularia* sp. as substrate. The number of replicates *per* density varied from 7 to 9. All individuals in each experimental unit were weighted to 0.1 mg every 30 d after being hand dried with paper napkins. Although size rather than weight is the usual parameter, we used weight because of the small size of individuals and the difficulties in accurately measuring the smallest juveniles. The correlation between total length (TL, measured from the tip of the rostrum to the posterior end of the telson and weight was equation: weight = 0.019 $TL^{2.83}$, r^2 = 0.98 for females and weight = 0.03 $TL^{2.61}$, r^2 = 0.99 for males (KDC and LSLG, unpublished). Total grow-out period lasted 90 d, therefore individual shrimp were weighted at days 30, 60, and 90. Food was provided *ad libitum* to avoid feeding constraints (4% of food of the replicate's total weight). The presence of ovigerous females was also recorded, and offspring born during the experiment were removed to prevent changes in density. Water was completely replaced once a week. Experimental animals were frozen at –20 °C at the end of the experimental period for biochemical analysis.

Total glycogen, lipid, and protein were determined spectrophotometrically in homogenates of all individuals from each replicate, in agreement with the methods described by Folch *et al.* (1957), Van Handel (1965), and Bradford (1976), respectively, and modified by Tropea *et al.* (2015) for *N. davidi*. In cases in which biomass was not sufficient for testing, replicates were pooled. Females and males were analyzed separately. Number of replicates *per* treatment varied from four to six depending on the amount of biomass available.

Monoculture versus mixed culture

Gender segregation was evaluated by applying three rearing conditions: mixed culture, monosex culture with only females and monosex culture with only males (Experiment 2). For this purpose, recently hatched juveniles were placed in 8 l aquaria for 30 days or until they reached the proper size in which secondary sexual characters became evident, so that sexes could be easily determined under a stereomicroscope. Specimens were sexed based on the morphology of the first and second male pleopods (Barbier, 2010; Pantaleão *et al.*, 2015b). Individuals within 10 and 15 mg (mean wet weight of 12.5 ± 2.5 mg) were selected for the experiment. For the mixed culture, females and males were set at an equal ratio, whereas each replicate contained either all females or all males for the monosex culture. Juveniles were placed in 2 l aquaria, and randomly assigned to the treatments. Stocking density was 5 individual l^{-1} (with five replicates *per* treatment). Individual shrimp were wet weighted every 15 days, and the total growth period was 90 days. The presence of ovigerous females was also recorded, and offspring born during the experiment were removed to prevent changes in density and treatment.

Statistical analysis

The weight of shrimp over time was registered for both experiments. Because shrimp could not be individualized throughout the experiments, the mean weight of individuals from each experimental unit (replicate) was used as a response variable, and compared within each essay by using a general linear mixed model with a repeated measures design. The replicate was included as a random effect, and the fixed effect factors were density (for Experiment 1, effect of density), gender and treatment (for Experiment 2, monosex culture), and time (for both). Variance was modeled using varExp (Zuur *et al.*, 2010) to correct for the violation of the homogeneity of variance assumption (assessed by graphical tools according to Zuur *et al.*, 2010), and an autoregressive correlation of order 1 as a correlation structure (see Zuur *et al.*, 2009).

For Experiment 1, the final weight of males was analyzed with a general linear mixed model, considering density as a fixed effect factor, and replicate as a random effect. Final weight of females

was also analyzed with a general linear mixed model; however, the female condition (ovigerous *versus* non-ovigerous) was also regarded as a fixed factor. Residuals showed no patterns and adjusted for normality in both cases, there was therefore no need for transformation or further modeling. The biochemical composition of shrimp (glycogen, lipid, and protein contents) at the end of the growth period was compared through separate general linear models (with density, gender, and their interaction as fixed factors). In the case of lipid and protein content, generalized least squares (GLS) were employed to correct for heterogeneity in residuals using varPower as a function of variance (Zuur *et al.*, 2010).

To evaluate the effect of density on sex ratio (number of females over total number of shrimps per replicate at final time), a generalized linear model (GLM) was used, with a binomial distribution and "logit" as link function. Survival rate (percentage of live shrimp at the end of the experimental period) was tested separately for both experiments also with a GLM (Zuur *et al.*, 2010). The level of significance was set at 0.05. A *posteriori* comparisons were made using Tukey's test in all cases. All analyses were carried out using R Studio version 3.3.1 (R Core Team, 2015).

RESULTS

Effect of density

Shrimp stocked at $D_{2.5}$ and D_5 attained a larger weight than those from D_{10} (Fig. 1). At 30 days, individuals from all densities grew uniformly, but individuals from $D_{2.5}$ and D_5 presented a higher weight than D_{10} (density:time; $df = 4$; $F = 5.978$; $P = 0.0004$) at 60 days. This tendency prevailed until the end of the experiment (90 days) (Fig. 1, Table 1), when females from $D_{2.5}$ weighted 45% more than females stocked at D_{10} ($df = 2$, $F = 9.03$, $P = 0.0015$) (Fig. 2). Females from D_5 did not differ from the other densities. Ovigerous females were substantially larger (81.55 ± 3.02 mg) than non-ovigerous ones (74.81 ± 2.74 mg) regardless of density ($df = 1$, $F = 8.27$, $P = 0.0051$), mean values displayed therefore correspond to a global mean weight. Females became ovigerous after a two-month growth period and at all densities (data not shown). $D_{2.5}$ males attained a greater weight than those from the remaining densities ($df = 2$; $F = 13.27$; $P = 0.0004$) (Fig. 2), they were 29% heavier than in the other treatments. Survival rate was high at all densities (Table 2). Sex ratio was also unaffected by density, globally female: male odd ratio was 0.898 (Table 3).

Glycogen concentration was similar for females and males at all densities. Total lipid content varied among sexes and treatments. D_5 males had the lowest concentration of lipids, whereas D_5 and D_{10} females had the highest amounts. D_5, D_{10} females, and $D_{2.5}$ males had the highest protein content, whereas the remaining groups had the lowest content (Table 4).

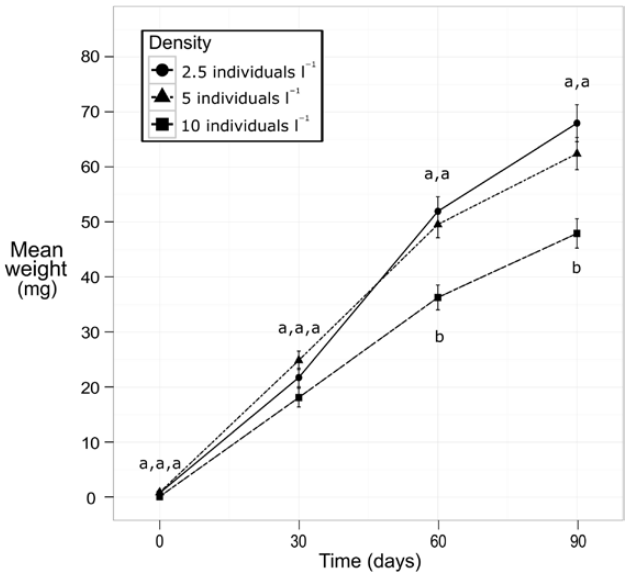

Figure 1. Mean body weight of *Neocaridina davidi* during 90-days growth period at three stocking densities ($D_{2.5}$, D_5, and D_{10} individuals^{-1}). Different letters represent statistically significant differences among treatments at each time ($P < 0.05$).

Table 1. Parameter estimates (regression coefficients) of fixed factors from the repeated measures multiple regression testing the effect of density ($D_{2.5}$, D_5, and D_{10} individuals l^{-1}) on the growth of *Neocaridina davidi* over time. Parameter estimates were calculated as contrasts with the intercept: "D_{10}" and "Day 30". The level of significance was set at 0.05.

Factor	Estimates	Standard Error	*t*	*P*
(Intercept)	18.10	1.72	10.54	< 0.0001
$D_{2.5}$	3.65	2.4	1.51	0.1356
D_5	6.76	2.40	2.82	0.0064
Day 60	18.19	1.78	10.25	< 0.0001
Day 90	29.84	2.54	11.74	< 0.0001
$D_{2.5}$ x Day 60	12.00	2.72	4.41	< 0.0001
D_5 x Day 60	6.52	2.59	2.52	0.0144
$D_{2.5}$ x Day 90	16.38	4.02	4.08	0.0001
D_5 x Day 90	7.74	3.73	2.07	0.0423

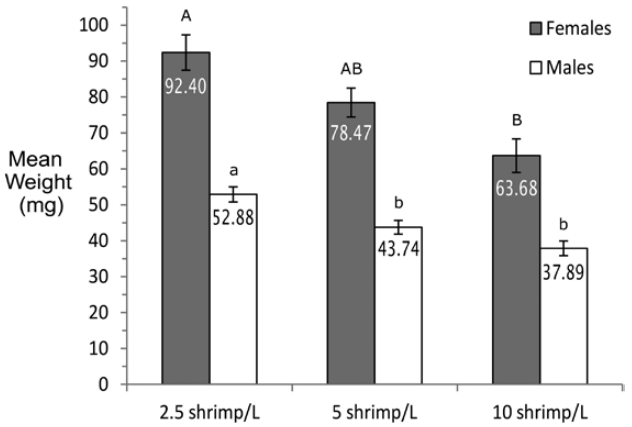

Figure 2. Female and male final mean body weight at the end of the growth period for Experiment 1 (90 days). Mean (± standard error) weight (mg) of adult *Neocaridina davidi* individuals cultured at different stocking densities ($D_{2.5}$, D_5, and D_{10} individuals l^{-1}). Comparisons among densities were made in the same gender, and are shown in the superscripts, those in capital letters correspond to females, those in lower case to males. Treatments without a common superscript differ ($P < 0.05$).

Table 2. Effect of density on the survival of *Neocaridina davidi* at 90 days. The survival rates of shrimp are expressed as percentage and were estimated from the logistic regression coefficients after applying the inverse logit function to transform parameters to their natural scale. Treatments without a common superscript differ ($P < 0.05$).

Density	Mean survival (%)	Confidence interval	Number of replicates
$D_{2.5}$	92.50 ^{a}	(79.20 – 97.56)	8
D_5	93.33 ^{a}	(85.95 – 96.97)	9
D_{10}	87.14 ^{a}	(87.14 – 91.75)	7

Monoculture versus mixed culture

No differences were found among treatments at day 15. Females (both from mixed and monoculture treatments) had a larger mean weight than males ($P < 0.05$). This difference was maintained throughout the entire experiment. Monosex culture showed no disparity from mixed culture at any time (Fig. 3, Table 5). Survival rate was also unaffected by treatment and gender (Table 6). Ovigerous females were recorded at approximately 45 days after the start of the experience.

DISCUSSION

Achieving harvest size at a faster rate is one of the main goals of commercial aquaculture hence if survival, health, and growth are not compromised, the higher the stocking density, the higher the profits. Optimal stocking density will depend on the species although external factors such as temperature, water quality, or feeding, can affect this culture parameter (Baskerville-Bridges & Kling, 2000).

Adult mean weight (females and males) of *Neocaridina davidi* was significantly higher in those stocked at $D_{2.5}$ and D_5 in Experiment 1. Intraspecific competition occurs when two or more individuals from the same species compete for a limited resource such as food, shelter, space, or any other factor crucial for reproduction or survival. In *Macrobrachium amazonicum* (Heller, 1862) competition is known to heighten when such resources become insufficient (Moraes-Valenti *et al.*, 2010). Because food availability was *ad libitum* and similar for all treatments, growth constraints were probably due to a lack of physical space. As density increases, space can become a limiting factor. This inhibition became evident in *N. davidi* after 30 days of growth.

Ovigerous females were larger than non-ovigerous females at all densities. In some gonochoric species such as the caridean shrimp *Hippolyte williamsi* Schmitt, 1924, males were smaller than females, and also ovigerous females were larger than non-ovigerous females (Espinoza-Fuenzalida *et al.*, 2008) as in our results. Females of *N. davidi* stocked at $D_{2.5}$ and D_5 were approximately 45% larger than those stocked at D_{10}. Tropea & López-Greco (2015) found a positive correlation between realized fecundity (number of eggs *per* spawn *per* female) and body weight in females of *N. davidi*, indicating that, as expected, larger females produce a larger number of eggs. Even though no relationship was found between actual fecundity (number of newly hatched juveniles per female) and female body size (Tropea & López-Greco, 2015; Tropea *et al.*, 2015), larger individuals are often more colorful and conspicuous, therefore breeding larger females could be favorable for the producer. In contrast, males stocked at $D_{2.5}$ reached a greater mean weight, proving that low densities favored higher growth rates.

Sexual differentiation in *N. davidi* was not affected by stocking density and showed that sex ratio was slightly biased towards males. Sex ratio is usually inclined towards the gender with the least fertility values, according to mathematical models (Charnov & Bull, 1989). Bias among sexes and the strong sexual dimorphism may therefore be consequences of the asymmetrical energetic investment for females and males, given that the former expends more resources for the synthesis of gametes (see Charnov & Bull, 1989).

In species with a high degree of aggressiveness, such as the caridean shrimps *Macrobrachium rosenbergii* and *M. australiense* Holthuis, 1950, males have hypertrophied cheliped that are used as weapons and actively compete for access to females. Dominant males often prevent smaller males from reproduction (Bauer, 2004). In contrast, *N. davidi* is a gregarious species lacking enlarged chelipeds, therefore direct competition for females is not likely to occur. Sexual size dimorphism in this species was also assessed by Sganga *et al.* (2016), who showed that not only adult females are larger than males, but also present morphometric differences in size of the carapace and rostrum than males. *Neocaridina davidi* has three characteristics that are consistent with a "pure search" mating system, as described by Wickler & Seibt (1981): populations with high densities of individuals, absence of cheliped weaponry, and sexual dimorphism in terms of size. Male mating success depends on their capacity to locate and copulate with females, and involves no courtship or agonist encounters between males (Correa & Thiel, 2003). Smaller males are more agile and therefore more efficient at finding females (Correa & Thiel, 2003; Rasch & Bauer, 2016). Another caridean, *Palaemon pugio* (Holthuis, 1949) presents a similar reproductive behavior as *N. davidi*, and females are also larger than males (Bauer & Abdalla, 2001). Both *N. davidi* and *P. pugio* are non-aggressive species and possess small chelipeds in both sexes. Reproductive competition occurs indirectly, a reason why larger size or chelipeds have not been selected through the course of their evolutionary history (see Bauer, 2004). Taking this information into account, it is not surprising that gender segregation (Experiment 2) had no effect over growth in both sexes in *N. davidi*.

Survival rate was also unaffected by density or gender segregation, reinforcing the "non-aggressive nature" of *N. davidi*. The morphology of this species provides no evidence of hierarchical structures (between or among genders) or aggressive behavior under rearing conditions, making it more tolerant to living at higher stocking densities. Studies on *Palaemon varians* (Leach 1814), showed a similar tendency, given that density had no effect over shrimp survival or larval size (Palma *et al.*, 2009).

Table 3. Sex ratio of *Neocaridina davidi* according to density at the end of the growth period of 90 days. Female odd ratio is estimated as the proportion of females (π) over the proportion of males ($1 - \pi$). Treatments without a common superscript differ ($P < 0.05$).

Density	Mean proportion of females (%)	Confidence Interval	Female odd ratio (π/ 1– π)
$D_{2.5}$	40.5 [a]	(26.1 – 56.8)	0.682
D_5	54.8 [a]	(44.1 – 65.0)	1.211
D_{10}	44.3 [a]	(35.7 – 53.2)	0.794
Total	47.3 [a]	(41.1 – 60.9)	0.898

Table 4. Effect of density on the biochemical composition of *Neocaridina davidi* at the end of the experiment for the three densities tested ($D_{2.5}$, D_5, and D_{10} individuals.l^{-1}). Mean values are given (± SE). Numbers between brackets represent the number of replicates. Treatments without a common superscript within a row are significantly different ($P < 0.05$).

Gender	Female			Male		
Density (individuals^{-1})	$D_{2.5}$	D_5	D_{10}	$D_{2.5}$	D_5	D_{10}
Glycogen (ug/mg)	2.12 ± 0.25^{a}(5)	2.37 ± 0.23^{a}(6)	2.23 ± 0.26^{a}(4)	2.07 ± 0.26^{a}(4)	2.33 ± 0.24^{a}(5)	2.19 ± 0.23^{a}(6)
Lipid (ug/mg)	6.53 ± 1.49^{ab}(5)	15.01 ± 4.11^{b}(6)	20.06 ± 6.03^{b}(6)	9.06 ± 2.30^{ab}(5)	3.00 ± 0.48^{a}(6)	7.07 ± 1.66^{ab}(5)
Protein (ug/mg)	41.92 ± 5.61^{a}(5)	146.95 ± 23.47^{b}(3)	112.19 ± 14.11^{b}(5)	118.72 ± 19.21^{b}(3)	30.51 ± 3.80^{a}(6)	44.54 ± 5.93^{a}(5)

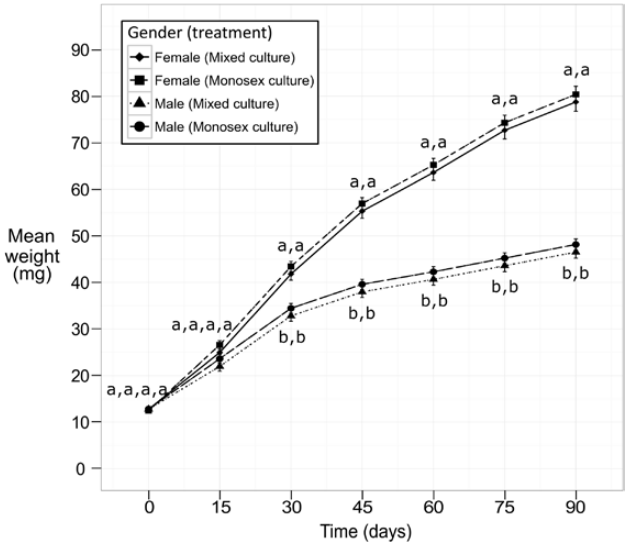

Figure 3. Mean body weight throughout the 90-day growth period according to treatments. Female and male *Neocaridina davidi* were stocked at a 5 individuals l^{-1} density and were grown in monosex cultures (genders stocked separately) and mixed cultures (both genders together). Different superscripts represent statistically significant differences among treatments at each time ($P < 0.05$).

Table 5. Parameter estimates (regression coefficients) of fixed factors from the repeated measured multiple regression, testing the effect of monosex culture *versus* mixed culture on the growth of *Neocaridina davidi* over time. Parameter estimates were calculated as contrasts with the intercept: "Mixed culture", "Female" and "Day 15".

Factor	Estimates	Standard Error	*t*	*P*
(Intercept)	24.94	1.11	22.41	< 0.00001
Monosex Culture	1.63	1.23	1.33	0.1861
Day 30	16.85	0.97	17.40	< 0.00001
Day 45	30.36	1.13	26.78	< 0.00001
Day 60	38.65	1.26	30.58	< 0.00001
Day 75	47.71	1.44	33.25	< 0.00001
Day 90	53.80	1.57	34.31	< 0.00001
Male	–2.97	0.87	–3.42	0.0009
Day 30 x Male	–6.01	1.29	–4.65	< 0.00001
Day 45 x Male	–14.37	1.44	–10.02	< 0.00001
Day 60 x Male	–19.98	1.54	–12.97	< 0.00001
Day 75 x Male	–26.11	1.68	–15.52	< 0.00001
Day 90 x Male	–29.26	1.80	–16.29	< 0.00001

Table 6. Female and male individuals of *Neocaridina davidi* survival rate for mixed culture and monoculture at the end of the growth period of 90 days. Survival rate is presented as percentage, and was estimated from the logistic regression coefficients after applying the inverse logit function to transform parameters to their natural scale. Treatments without a common superscript differ ($P < 0.05$).

Treatment	Gender	Mean survival (%)	Confidence interval	Number of replicates
Mixed culture	Female	82.90 [a]	(66.91 – 92.08)	5
Monoculture	Female	92.55 [a]	(83.73 – 96.77)	5
Mixed culture	Male	73.10 [a]	(55.36 – 85.62)	5
Monoculture	Male	87.45 [a]	(76.65 – 93.67)	5

Proteins were the most abundant biochemical components of *N. davidi*, followed by lipids and glycogen. These results are in agreement with previous studies on the species (Tropea *et al.*, 2015), as well as for the embryological states of another decapod, the crayfish, *Cherax quadricarinatus* (Martens, 1868) (García-Guerrero *et al.*, 2003), and the larval stages of other crustaceans (Racotta *et al.*, 2003). Many studies have analyzed the effect of different parameters such as water quality, diet, and temperature on the biochemical composition of crustaceans (*e.g.*, Dey *et al.*, 1993; Anger, 1998; Rodríguez-González *et al.*, 2006; Anger *et al.*, 2009; Weiss *et al.*, 2009; Cerón-Ortiz *et al.*, 2015; Tropea *et al.*, 2015), and other invertebrates (*e.g.*, Mercer *et al.*, 1993; Dunstan *et al.*, 1996; Fernandez, 1997), but to our knowledge, few have studied the effect of density (Montero *et al.*, 1999; Khan, 1994; Calado *et al.*, 2005a; Andrés *et al.*, 2007). Glycogen levels were unaffected by gender or density in *N. davidi*. Females kept at $D_{2.5}$ tended to have lower lipid and protein contents, although the difference was not statistically significant for the former. An analogous pattern was observed in the larvae of the brachyuran crab *Maja brachydactyla* Balss, 1922, where lipid and protein concentrations were higher in larvae reared at the highest density, with necrophagy suggested as a possible explanation (Andres *et al.*, 2007). Such a hypothesis, however, does not seem applicable for *N. davidi*. Triglyceride levels in the hemolymph of the brachyuran crab *Ocypode quadrata* (Fabricius, 1787) showed a decrease during spring, which coincides with the beginning of the reproductive period when the gonad initiates gametogenesis (Vinagre *et al.*, 2007). Many studies have determined that oogenesis represents a major energetic expense for females since lipids are the primary source of nutrients for the developing embryo, especially in lecitotrophic species such as *N. davidi* (see Mourente & Rodríguez, 1991; Palacios *et al.*, 2000). The results of our experiment could be a consequence of an increased reproductive frequency in females kept at the lowest density, although further research is needed to specifically address this point. Males at $D_{2.5}$ were larger than those at other densities, so it is not surprising that they had the highest protein content, given that the decapod pleon consists mainly of muscle fibers.

Increasing the stocking density showed a negative effect on the growth rate of *N. davidi* although survival rate remained unaffected. It is therefore recommended that shrimp should be stocked at a density of 2.5 shrimp l^{-1} to obtain larger females and males. Moreover, gender segregation is not necessary for optimal grow-out.

ACKNOWLEDGEMENTS

This study is part of NDV's undergraduate research at Universidad de Buenos Aires, Argentina. LSLG is grateful to Agencia Nacional de Promoción Científica y Tecnológica (PICT 2012 project 1333), Consejo Nacional de Investigaciones Científicas y Técnicas (PIP 2015–2017 project 11220150100544) and Universidad de Buenos Aires (2014- 2017 project 20020130100186BA) for supporting the research. We are also grateful to Adriana Pérez and Dr. Gerardo Cueto (Instituto de Ecología y Evolución de Buenos Aires) for their advice in the statistical analysis and software, and to Amir Dyzenchauz (Instituto de Biodiversidad y Biología Experimental y Aplicada) for the revision of the manuscript, and two anonymous reviewers.

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